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SHORT NOTE

Pollen Resources Stored in Nests of Wild Bees *Xylocopa ciliata* Burmeister and *Megachile pusilla* Pérez (Hymenoptera: Anthophila) in a Temperate Grassland-Forest Matrix

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Abstract

Pollen analysis was employed to study the diet composition of two wild bees in a patch of temperate grassland invaded by exotic plants. Thirty pollen types from 14 families and two unidentified types were present in the seven samples analyzed. The three samples from *Megachile pusilla* Pérez were composed of 100% *Lotus glaber* and the four samples from *Xylocopa ciliata* Burmeister of abundant pollen (>5%) of *Lotus glaber*, *Galega officinalis*, *Adesmia bicolor* and type *Senna-Chamaecrista* (all Fabaceae). The latter resource likely foraged out of the park, which could be an evidence to support its pollen preference for Fabaceae. However, a larger number of samples is necessary to identify the specialization status of *X. ciliata*. The preference for *Lotus* in this site was due to temporal specialization as *M. pusilla* was identified as *polylectic* in its origin area, and this could help to explain its effective naturalization in the New World and other areas of the Old World.

Pollen analysis of nest provisions is a useful tool to identify their botanical origin and to understand the pollen specialization in bees (Cane & Sipes, 2006; Müller & Kuhlmann, 2008). Even extreme pollen specialists can take nectar from many floral species but forage pollen on a reduced number of host-plants (Robertson, 1925; 1926; Cane & Sipes, 2006; Minckley & Roulston, 2006; Müller & Kuhlmann, 2008; Vossler, 2013; 2014). Threshold values of 10 and 5% total pollen counts have commonly been used to distinguish minor or potential contaminants from abundant pollen types (Ramalho et al., 1985; Kleinert-Giovannini & Imperatriz-Fonseca, 1987; Cane & Sipes, 2006; Müller & Kuhlmann, 2008) which is necessary to correctly identify pollen specialization even in polylectic bees (Vossler, 2018).

The aim of the present study was to identify the botanical origin of nest pollen provisions of two wild bees (the exotic *Megachile pusilla* Pérez and the neotropical *Xylocopa ciliata* Burmeister) and their pollen specialization at a same site during the period of nesting activity. It is hypothesized that both species are polylectic, similar to most species of *Megachile* and *Xylocopa*.

M. pusilla was found nesting in small soil cracks of a bank and *X. ciliata* in internodes of only large stems of the invasive *Dipsacus fullonum* L. (Fig 1). Their nests were hard to find in the site sampled and no studies on nest pollen provisions were performed for *M. pusilla* in areas where it was naturalized neither for *X. ciliata*. Nests were provisioned during summer and sampled during summer and fall (three nests of *M. pusilla* sampled on 12th February 2011 and two samples from nest 1 (nest 1A and B) of *X. ciliata* on 27th December 2008 and one sample (nest 1C) from 1st January 2009 and one sample from nest 2 in June 2012) (Table 1; Fig 1). The study was carried out at the Parque Ecológico Municipal de La Plata (34° 51'-52' S; 58° 03'-05' W), a 200 ha patch of temperate grassland composed of many melittophilous species native to the Oriental district of the Pampean region *sensu* Cabrera (1971) but heavily colonized by exotic plants (Vossler et al., unpublished data), at Villa Elisa city, Buenos Aires province, Argentina.

The whole pollen provisions from nest cells of *M. pusilla* and dry feces of post-defecating larvae from nest



entrances of *X. ciliata* were sampled and stored in plastic tubes at 5 °C. In the laboratory, they were dissolved in distilled water at 80-90 °C for 10-15 minutes, pressed when necessary using a glass rod, stirred by hand for a few minutes, and filtered. Finally, to obtain pollen sediment, samples were centrifuged at 472 x g for 5 minutes, and acetolized (Erdtman, 1960). Pollen types were identified at 400 and 1,000 x magnification and 500 grains per slide were counted (except in sample 1A as only 204 grains were found) using a light microscope Leitz Laborlux. Pollen grain identification was carried out comparing nest pollen grains with reference

pollen. The reference pollen collection was made from flowers of plant species mainly collected in Parque Ecológico Municipal de La Plata (Villa Elisa city) and La Plata city, in the northeastern of Buenos Aires province, Argentina. These plant specimens were pressed, dried, identified by the author and deposited in the Herbarium Lorentz (DTE) of Diamante, Entre Ríos, Argentina. The vegetation and flowering of the site was quantitatively recorded from August 2008 to August 2009 (Vossler et al., unpublished data). To identify pollen specialization categories, the lexica of Cane and Sipes (2006) and Müller and Kuhlmann (2008) were applied.

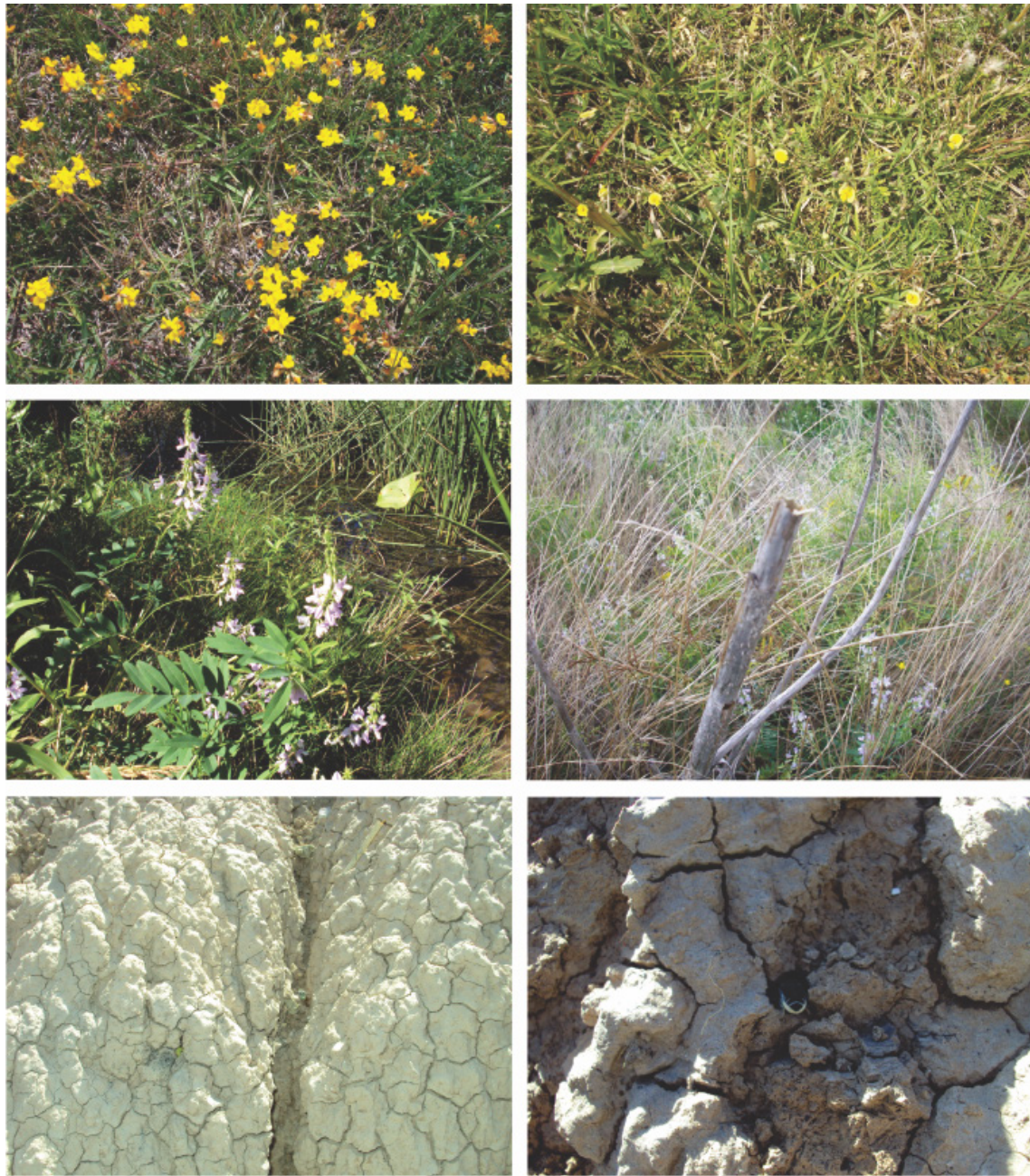


Fig 1. Pollen host blooming: A_ *Lotus glaber*; B_ *Adesmia bicolor*; C_ *Galega officinalis*. Nesting sites of *Xylocopa ciliata* (D) and *Megachile pusilla* (E, F): D_ Nest 1 of *Xylocopa ciliata* in a thick stem of *Dipsacus fullonum* surrounded by *Galega officinalis* and *Lotus glaber* during blooming; E_ Leaf fragments in a completed nest cell (left) and transported by a female for nest building (right); F_ Scopal pollen of *Megachile pusilla* during nest provisioning.

A total of 30 pollen types from 14 families and two unidentified types were present in the seven samples analyzed (Table 1). From them, most were minor pollen ($\leq 5\%$) and only four types from two subfamilies of Fabaceae were abundant: *Lotus glaber*, *Galega officinalis*, *Adesmia bicolor* and type *Senna-Chamaecrista* (Table 1; Fig 2). The three samples of *M. pusilla* were composed of 100% *L. glaber*. The four samples of *X. ciliata* were composed of abundant pollen of the four Fabaceae in different representation and many minor pollen from many families (Table 1; Fig 2).

The fact that only *L. glaber* was identified composing the diet of *M. pusilla* would indicate strong specialization

on this species. However, as all the samples were from the same day, this result could be related to resources availability. Moreover, pollen analysis of 24 loads from 18 localities reported *Lotus* as abundant pollen and many families as hosts of *M. pusilla* in its area of origin, which is around the Mediterranean Sea in the Old World (Soltani et al., 2017), suggesting *polylecty*. Therefore, the preference for *Lotus* in this site was interpreted as temporal specialization. Its *polylectism* could help to explain its effective naturalization in the New World and other areas of the Old World.

An intensive usage of *Lotus* and other three genera in disparate tribes of Fabaceae was observed in *X. ciliata*.

Table 1. Abundance (%) of the pollen types found in *Xylocopa ciliata* and *Megachile pusilla* nest pollen samples, in alphabetic order of their families. The abundant resources ($> 5\%$) are shown in bold. ‘+’ includes pollen types present in the slides but not recorded during the counting.

Plant family	Pollen type	<i>Xylocopa ciliata</i>			<i>Megachile pusilla</i>			
		nest 1 A	nest 1 B	nest 1 C	nest 2	nest 1	nest 2	nest 3
Amaryllidaceae	<i>Zephyrantes minima</i>			+				
Apiaceae	<i>Eryngium</i>				0.39			
Apiaceae	type <i>Ammi</i>		+					
Arecaceae	Arecaceae						+	
Asteraceae, Astereae	<i>Grindelia pulchella</i>		+		2.39			
Asteraceae, Astereae	type <i>Baccharis</i>		+		1.19			
Asteraceae, Cardueae	<i>Carduus acanthoides</i>				1.99			
Asteraceae, Cardueae	<i>Cirsium vulgare</i>			+	4.58			
Asteraceae, Eupatorieae	<i>Eupatorium buniifolium</i>				0.39			
Asteraceae, Heliantheae	<i>Ambrosia tenuifolia</i>						+	
Asteraceae, Lactuceae	<i>Picris echioides</i>			+			+	
Asteraceae, Lactuceae	type <i>Hypochaeris</i>		+	1.86				
Asteraceae, Vernonieae	<i>Vernonia</i>			+	1.39			
Brassicaceae	type <i>Nasturtium</i>						+	
Caprifoliaceae	<i>Lonicera japonica</i>		+				+	
Casuarinaceae	<i>Casuarina</i>				+			
Celtidaceae	<i>Celtis</i>						+	+
Dipsacaceae	<i>Dipsacus fullonum</i>			+			+	
Fabaceae, Caesalpinioideae	<i>Gleditsia triacanthos</i>		0.15				+	+
Fabaceae, Caesalpinioideae	type <i>Senna-Chamaecrista</i>				22.91			
Fabaceae, Papilionoideae	<i>Adesmia bicolor</i>			32.94	0.79	+		
Fabaceae, Papilionoideae	<i>Galega officinalis</i>	28.43	49.84	56.19	22.51		+	
Fabaceae, Papilionoideae	<i>Lotus glaber</i>	69.12	49.84	7.81	40.04	100	100	100
Fabaceae, Papilionoideae	<i>Vicia sativa</i>	1.47	+	+				
Myrtaceae	<i>Eucalyptus</i>			+				
Poaceae	Poaceae type 1		+		0.79	+		+
Poaceae	Poaceae type 2	0.98	+					
Solanaceae	<i>Salpichroa organifolia</i>				0.20			
Solanaceae	<i>Solanum</i>			1.18				
Verbenaceae	<i>Verbena</i>		0.15					
Unidentified	Unidentified 1 (4-colporate psilate)						+	
Unidentified	Unidentified 2 (monosulcate)				0.39			

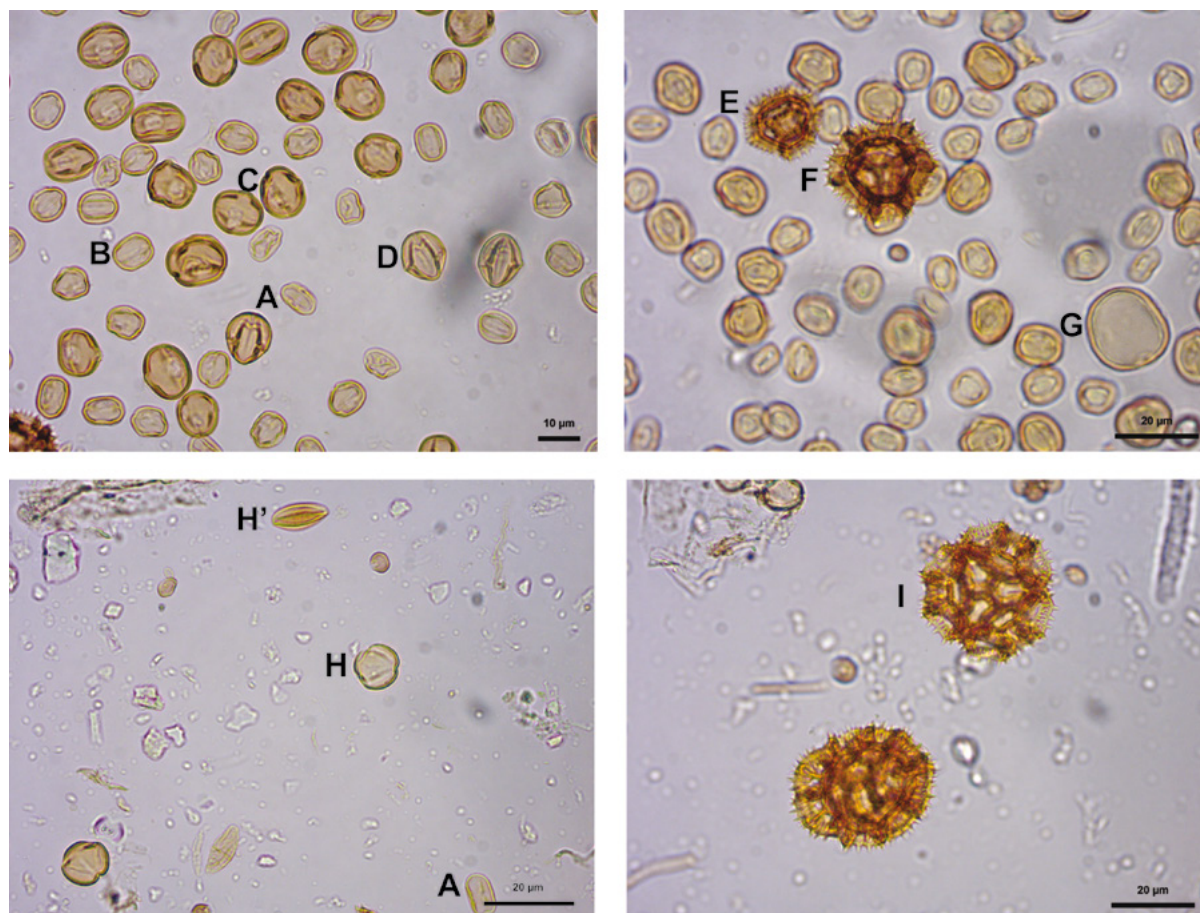


Fig 2. Abundant (A, B, C, and H) and minor (D, E, F, G, I) pollen types identified from nests of *Xylocopa ciliata*, seen under light microscope. A_ *Lotus glaber*; B_ *Galega officinalis*; C_ *Adesmia bicolor*; D_ *Solanum*; E_ type *Hypochaeris*; F_ *Picris echinoides*; G_ Poaceae type 1; H and H'_ type *Senna-Chamaecrista* (H': collapsed grain); I_ *Vernonia*.

The high abundance of Fabaceae in only four samples could be considered as a strong evidence of pollen specialization on the whole family: *oligolecty* (*sensu* Cane & Sipes, 2006) or *broad oligolecty* (*sensu* Müller & Kuhlmann, 2008). Furthermore, as vegetation was recorded in this site where nests were sampled, the pollen types foraged could be ascribed to the plant species found within this area. Moreover, this fact allowed for the identification of the abundantly foraged type *Senna-Chamaecrista* as not belonging to this natural plant community and likely belonging to an ornamental species cultivated in the urbanized landscape surrounding this park and therefore foraged out of the park. This fact could be a further evidence to support its pollen preference for Fabaceae.

Palynological studies have not yet been carried out in this *Xylocopa* species. Some of the records on floral resources seem to suggest *polylecty* (Sakagami et al., 1967; Sakagami & Laroca, 1971; Hurd, 1978; Schlindwein, 1998; Schlindwein et al., 2003; Gonçalves & Melo, 2005; Dalmazzo, 2010). However, the differentiation between males and females as well as nectar and pollen intake, necessary to identify the pollen specialization status (Cane & Sipes, 2006; Müller & Kuhlmann, 2008; Vossler, 2013; 2014; 2018), was not done in most of these studies, but the fact that *Solanum* has pollen-only flowers (Vogel, 1978; Buchmann, 1983) suggests that it

is a legitimate pollen host of *X. ciliata*. The study of a larger number of nest pollen samples is necessary to identify the specialization status of *X. ciliata* and would likely include *Solanum* as an abundant pollen host.

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